IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

HARDERN et al

Serial No. **09/508,195**

Filed: **March 8, 2000**

For: **NOVEL COMPOUNDS**

Atty. Ref.: **3764-2**

Group: **1624**

Examiner: **Ford, J.**

* *

Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

DECLARATION

Sir:

I, Robert J. Riley, do hereby declare and state as follows:

1) I have been employed by AstraZeneca UK Limited and its predecessor companies for ten years. I now work within the Department of Physical and Metabolic Sciences as a Principal Research Scientist, supervising thirteen laboratory workers. I received my undergraduate degree (BSc Hons) from the University of Liverpool in 1986 and my PhD from the University of Liverpool in 1989. My technical expertise is in the field of Drug Metabolism and Pharmacokinetics.

2) I am aware of both US patent number 6,251,910 (Guile *et al*) and US patent application number 09/508,195 (Hardern *et al*) that each disclose compounds which act as P_{2T}-receptor antagonists. These compounds may be used as pharmaceutical agents for inhibition of platelet aggregation. Both the patent (Guile *et al*) and the patent application (Hardern *et al*) have been assigned to AstraZeneca UK Limited. I am familiar with the compounds disclosed and I have been involved in testing and analyzing their biological activity.

3) The following factors are among those important in a compound having a low predicted dose in man:

   i) Good potency at the human receptor;

   ii) Good metabolic stability in man.

   Therefore increasing metabolic stability while maintaining affinity for the P_{2T}-receptor will result in effective treatment at a lower predicted human dose.

4) The compounds exemplified in Hardern *et al* are predicted to be active at a lower dose in man than those in Guile *et al* due to an unexpected combination of good potency at the P_{2T}-receptor and good human metabolic stability *in vitro*.

5) Good potency is defined as an affinity for the P_{2T}-receptor greater than 8 in the ligand binding assay described in the pharmacological data below.

6) It is well documented in the literature that *in vitro* measurements may be used to predict the *in vivo* metabolic stability of drugs. For example, this issue is discussed in the following papers:

   i) A D Rodrigues, Biochem. Pharmacol., 1994, 48, 2147

   ii) M Mistry, J B Houston, Drug Metab. Dispos.' 1987, 15, 710

   iii) J B Houston, Biochem. Pharmacol., 1994, 47, 1469

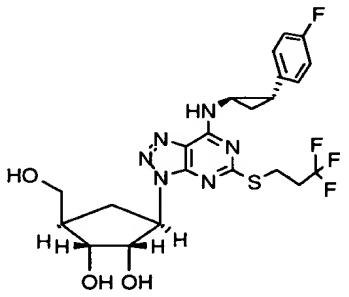
7) *In vitro* studies in human *hepatic* microsomes showed that compounds of the general structure exemplified in Guile *et al* are metabolised by oxidation and glucuronidation. The compounds exemplified in Hardern *et al* display the unexpected advantage of being more metabolically stable to both oxidation and glucuronidation. Since the compounds also maintain P_{2T}-receptor affinity the predicted therapeutic dose for inhibition of platelet aggregation in man shows advantage.

8) The rate of oxidation is measured by comparing the rate of oxidation of a test compound in human microsomes to a standard, dextromethorphan, a compound known to be rapidly metabolically cleared in man by oxidation (Martindale 23rd Edition, Pharmaceutical Press, 2002, p 1087). Therefore the higher the ratio the more metabolically stable the test compound.

9) The rate of glucuronidation is measured by comparing the rate of glucuronidation of a test compound in an *in vitro* glucuronosyltransferase assay to a standard, zileuton, a compound known to be rapidly metabolically cleared in man by glucuronidation (W M Awni etal. Clin. Pharmacokinet., 1995, 29 (suppl. 2) 49). Therefore the higher the ratio the more metabolically stable the test compound.

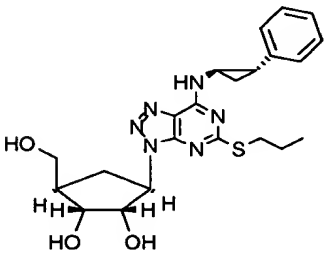
10) For the compounds exemplified in Hardern *et al* it can be shown that these compounds demonstrate the unexpected advantage of being active at a lower predicted dose in man as a result of a combination of increased metabolic stability together with high affinity for the P_{2T}-receptor.

11) The following compound is illustrated in Example 1 of Hardern *et al*:

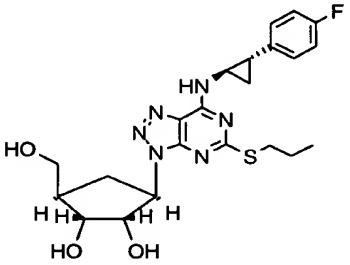


The following two compounds are exemplified in Guile *et* al:

Example 1



Example 61

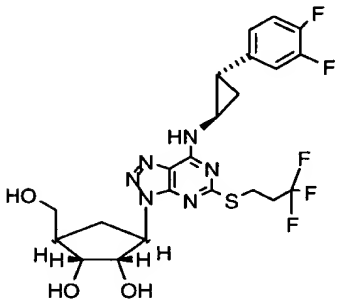


Of all the compounds exemplified in Guile *et al*, these two compounds are structurally closest to the compound exemplified in Hardern *et al* Example 1.

12) The data given in the table below shows that the Example 1 compound of Hardern *et al* maintains P_{2T} potency but displays unexpectedly higher metabolic stability to glucuronidation than the closest analogues in Guile *et al.*

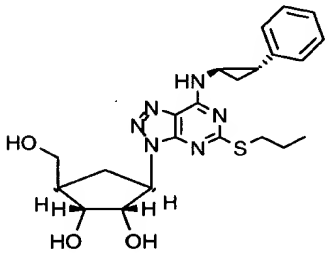
Example No	**US Patent or Application**	**P_{2T}-potency (Ki)**	**Human microsomes-stability to oxidation ratio to dex.**	**Human *in vitro* glucuronosyltransferase assay – stability to glucuronidation relative to zileuton**
Example 1	Hardern *et al*	8.4	Stable – no oxidation detected	27.8
Example 1	Guile *et al*	8.5	Stable	4.8
Example 61	Guile *et al*	8.3	>30	8.5

13) The following compound is illustrated in Example 2 of Hardern *et al*:

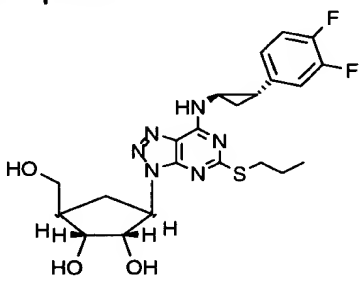


The following two compounds are the structurally closest examples in Guile *et al:*

Example 1



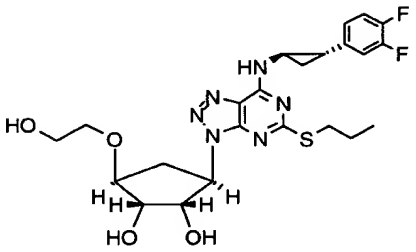
Example 69



14) The data given in the table below shows that the Example 2 compound of Hardern *et al* maintains P_{2T} potency but displays significantly higher metabolic stability to glucuronidation when compared to the closest analogues in Guile *et al.*

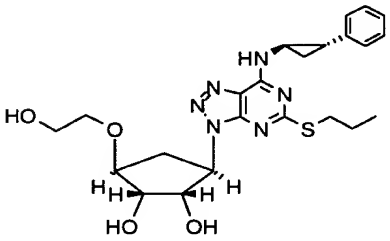
Example No	**US Patent or Application**	**P_{2T}-potency (Ki)**	**Human microsomes-stability to oxidation ratio to dex.**	**Human *in vitro* glucuronosyltransferase assay – stability to glucuronidation relative to zileuton**
Example 2	Hardern *et al*	8.3	Stable – no oxidation detected	Stable- no glucuronide detected
Example 1	Guile *et al*	8.5	Stable	4.8
Example 69	Guile *et al*	8.7	Stable	10.9

15) The following compound is illustrated in Example 3 of Hardern *et al*:

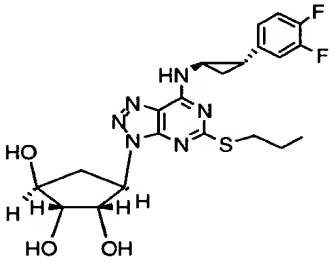


The following two compounds are the structurally closest examples in Guile *et al:*

Example 32



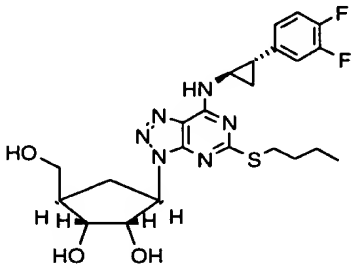
Example 68



16) The data given in the table below shows that the Example 3 compound of Hardern *et al* maintains P_{2T} potency but displays significantly higher metabolic stability to glucuronidation when compared to the closest analogues in Guile *et al*.

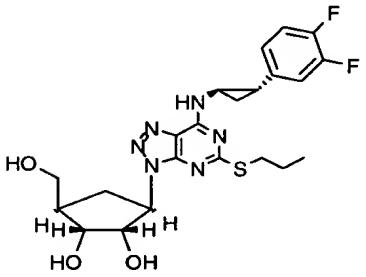
Example No	**US Patent or Application**	**P_{2T}-potency (Ki)**	**Human microsomes - stability to oxidation ratio to dex.**	**Human *in vitro* glucuronosyltransferase assay – stability to glucuronidation relative to zileuton**
Example 3	Hardern *et al*	8.7	24	Stable- no glucuronide detected
Example 32	Guile *et al*	8.3	13	24
Example 68	Guile *et al*	8.6	stable	3.9

17) The following compound is illustrated in Example 4 of Hardern *et al*:



The following compound is the structurally closest example in Guile *et al*:

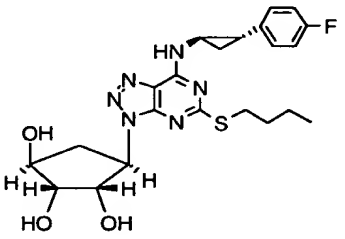
Example 69



18) The data given in the table below shows that the Example 4 compound of Hardern *et al* maintains P_{2T} potency but displays significantly higher metabolic stability to glucuronidation when compared to the closest analogue in Guile *et al*.

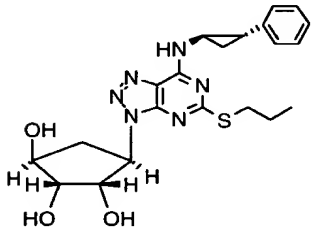
Example No	**US Patent or Application**	**P_{2T}-potency (Ki)**	**Human microsomes - stability to oxidation ratio to dex.**	**Human *in vitro* glucuronosyltransferase assay – stability to glucuronidation relative to zileuton**
Example 4	Hardern *et al*	8.3	29	42.8
Example 69	Guile *et al*	8.7	Stable	10.9

19) The following compound is illustrated in Example 5 of Hardern *et al*:

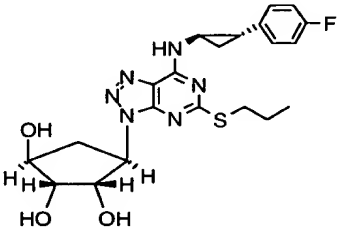


The following two compounds are the structurally closest examples in Guile *et al:*

Example 12



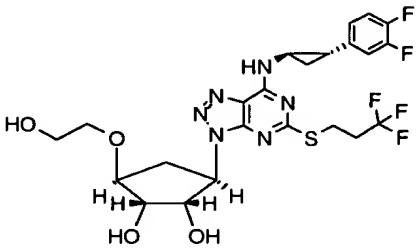
Example 19

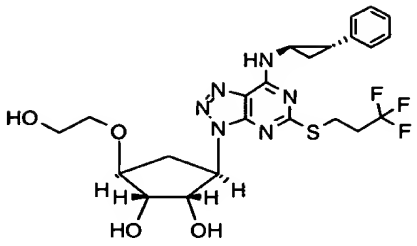


20) The data given in the table below shows that the Example 5 compound of Hardern *et al* maintains P_{2T} potency but displays significantly higher metabolic stability to glucuronidation when compared to the closest analogues in Guile *et al.*

Example No	**US Patent or Application**	**P_{2T}-potency (Ki)**	**Human microsomes - stability to oxidation ratio to dex.**	**Human *in vitro* glucuronosyltransferase assay – stability to glucuronidation relative to zileuton**
Example 5	Hardern *et al*	8.4	>30	Stable – no glucuronide detected
Example 12	Guile *et al*	8.6	Stable	2.7
Example 19	Guile *et al*	8.6	10.3	7.6

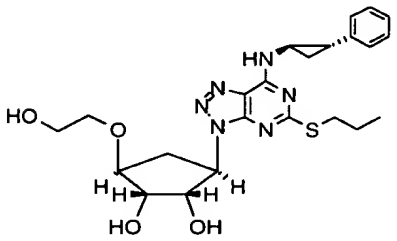
21) The following compounds are illustrated in Examples 6 and 7 of Hardern *et al*:



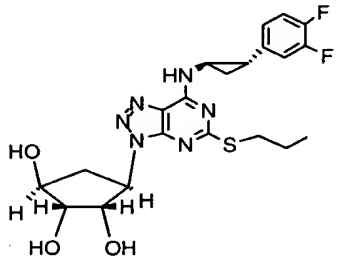


The following two compounds are the structurally closest examples in Guile *et al:*

Example 32



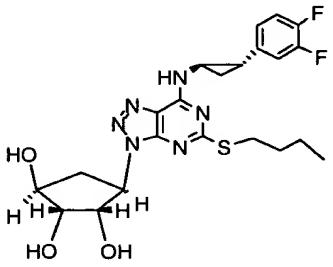
Example 68



22) The data given in the table below shows that the Example 6 compound and the Example 7 compound of Hardern *et al* maintain P_{2T} potency but display significantly higher metabolic stability to glucuronidation when compared to the closest analogues in Guile *et al*.

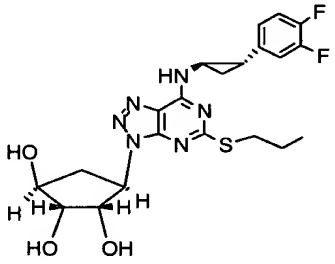
Example No	**US Patent or Application**	**P_{2T}- potency (Ki)**	**Human microsomes - stability to oxidation ratio to dex.**	**Human *in vitro* glucuronosyltransferase assay – stability to glucuronidation relative to zileuton**
Example 6	Hardern *et al*	9.0	31.8	Stable – no glucuronide detected
Example 7	Hardern *et al*	8.7	>24	50
Example 32	Guile *et al*	8.3	13	23.8
Example 68	Guile *et al*	8.6	stable	3.9

23) The following compound is illustrated in Example 8 of Hardern *et al*:

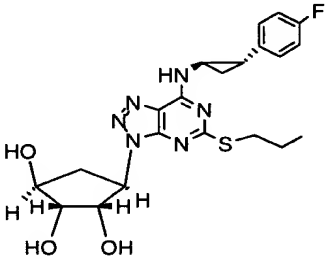


The following three compounds are the structurally closest examples in Guile *et al:*

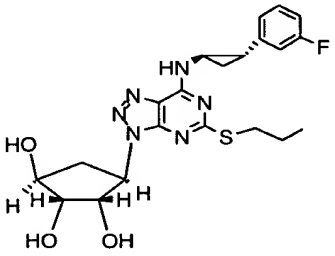
Example 68



Example 19



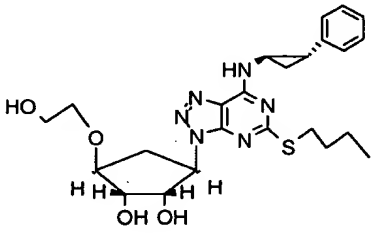
Example 99



24) The data given in the table below shows that the Example 8 compound of Hardern *et al* maintains P_{2T} potency but displays significantly higher metabolic stability to glucuronidation when compared to the closest analogues in Guile *et al*.

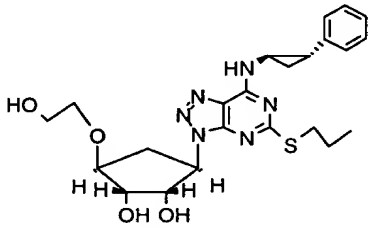
Example No	**US Patent or Application**	**P_{2T}-potency (Ki)**	**Human microsomes - stability to oxidation ratio to dex.**	**Human *in vitro* glucuronosyltransferase assay – stability to glucuronidation relative to zileuton**
Example 8	Hardern *et al*	8.4	>25	>20
Example 68	Guile *et al*	8.6	stable	3.9
Example 19	Guile *et al*	8.6	10.3	7.6
Example 99	Guile *et al*	8.8	stable	3.5

25) The following compound is illustrated in Example 9 of Hardern *et al*:



The following compound is the structurally closest example in Guile *et al:*

Example 32



26) The data given in the table below shows that the Example 9 compound of Hardern *et al* maintains P_{2T} potency but displays significantly higher metabolic stability to oxidation when compared to the closest analogues in Guile *et al.*

Example No	**US Patent or Application**	**P_{2T}-potency (Ki)**	**Human microsomes - stability to oxidation ratio to dex.**	**Human *in vitro* glucuronosyltransferase assay – stability to glucuronidation relative to zileuton**
Example 9	Hardern *et al*	8.5	>25	26
Example 32	Guile *et al*	8.3	13	23.8

I declare that all statements herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Robert J Riley

31st July, 2002
Date